Glass wool as a potential source of artifacts in chromatography

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During the course of the development of a micro method for the determination of unesterified fatty acids, it was observed that the glass wool used as a support for the column material in a chromatographic tube gave rise to a number of neutral and acidic constituents which seriously interfered with both qualitative and quantitative analysis by gas–liquid chromatography (GLC). In view of the widespread use of glass wool in laboratory operations, it was surprising to find no literature reference pertinent to our observation. Therefore, our experiences might be of interest to others, particularly those involved in trace analysis of organic constituents in natural products.

MATERIALS AND METHODS*

Glass wool was Corning No. 3950; different lots produced in various years (from 1964 to 1972) were obtained from laboratories in this Center, and the most recent lot (July 1976) was purchased through the A. H. Thomas Co., Philadelphia, Pa., U.S.A. Samples for analysis were taken with forceps from the innermost portion of the roll, and the wool was subsequently handled only with forceps. Disposable Pasteur Super pipets, 10 mm I.D. × 14.3 cm (Matheson Scientific, Moorestown, N.J., U.S.A.), were used as columns. Pentane (American Mineral Spirits, Conshohocken, Pa., U.S.A.) was purified by passing 4 l over a column layered from bottom to top with 25 g of neutral alumina (Brockmann Activity I), 25 g Hy Flo Super-Cel (Fisher Scientific, King of Prussia, Pa., U.S.A.), and 50 g of Hy Flo Super-Cel ground thoroughly in a mortar with 25 ml of concentrated sulphuric acid. The Celite layers were tamped tightly. The flow-rate was *ca.* 2 ml/min and the first 50 ml was discarded. The scrubbed pentane was distilled, and the fraction boiling at 36–37° was collected; dichloromethane (Burdick and Jackson Labs, Muskegan, Mich., U.S.A.) was used as received, after it was established that 8% of the residue obtained from evaporation of 30 ml gave no peaks under the GLC conditions described below.

GLC was performed with a Hewlett-Packard 5750A instrument with a flame ionization detector. A 2.4 m × 0.3 cm silanized stainless-steel column packed with 7.5% stabilized ethyleneglycol adipate (EGA) and 2% phosphoric acid on Anakrom ABS (Analabs, North Haven, Conn., U.S.A.) was used. Separations were carried out

* Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

isothermally at 210° with a helium flow of 30 ml/min; injection block and detector temperatures were 250°. The range was set at 10 and the attenuation at ×4. All glassware was rinsed with acetone and pure pentane before use and controls were run omitting only the glass wool.

Examination of glass wool

Approximately 1 g of glass wool was tamped tightly in a column and treated with the following sequence of reagents: (a) pentane until GLC showed that no additional solutes were eluted (40 ml); (b) dichloromethane until no more solutes were present (30 ml); (c) hydrogen chloride* followed by 20 ml of pentane. Solvents were removed at room temperature under a stream of nitrogen and the residues were dissolved in 100 μl of carbon disulphide for GLC analysis.

RESULTS AND DISCUSSION

Figs. 1 and 2 are the chromatograms of 8% of the pentane (a) and dichloromethane (b) residues, respectively, obtained from 1.2 g of a glass wool manufactured in July 1976. The majority of the peaks in Fig. 1 are hydrocarbons and phthalate esters as determined by mass spectrometry, whereas those in Fig. 2 are unesterified acids. All other glass wool samples examined (*e.g.*, wools produced in 1964, 1968, 1970, 1971, 1972) gave patterns that were both qualitatively and quantitatively different, but all contained a large number of constituents. Although the glass wool of July 1976 did not contain significant amounts of organic acid salts (isolated with reagent

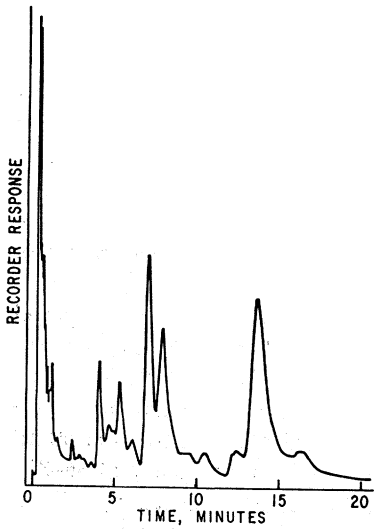

Fig. 1. GLC trace of 8% of the residue extracted with pentane from a glass wool produced in July 1976. Column, EGA–H_3PO_4, temperature 210°.

* The top portion of the column was inserted through a rubber stopper fitted into the pouring spout of a bottle of concentrated hydrochloric acid. The vapors were drawn through the glass wool for 1–2 min by pulling a slight vacuum on the other end of the column.

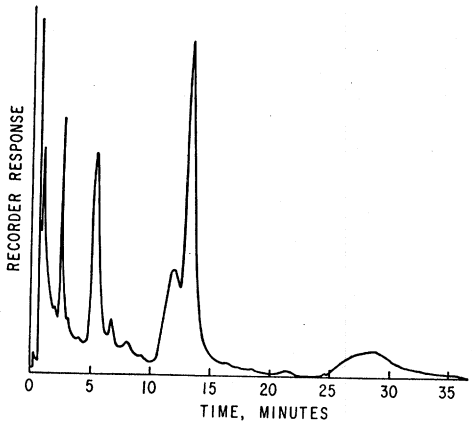

Fig. 2. GLC trace of 8% of the residue extracted with dichloromethane following exhaustive extraction with pentane of a glass wool produced in July 1976. Column, EGA–H_3PO_4, temperature 210°.

sequence (c)), in several of the other wools relatively large amounts of these salts were present and the acids were removable only after acidification of the wool.

The amount of organic matter on the glass wools that was extractable with pentane and dichloromethane varied from *ca.* 0.3 to 0.5 mg/g, with the bulk of the material being extractable with pentane.

It is clear from Figs. 1 and 2 that even small plugs of unpurified glass wool* can give rise to numerous artifacts under suitable conditions.

The most efficient way of eliminating the impurities detected in this study was to treat the wool for a few minutes with hydrogen chloride followed by continuous extraction in a Soxhlet for 24 h with dichloromethane. Other treatments, such as dichromate–sulphuric acid cleaning solution for 5 days at ambient temperature or at 100° for 16 h, or washing on a filter with 2 *N* hydrochloric acid, water, acetone, and pentane, failed to remove all of the impurities.

* The glass wool of July 1976 was selected for depicting the chromatograms of the extracts because it was the most recent lot available and the box had not been opened previously. Actually, it was one of the purest of the wools examined.